TABLE 2. *TRUS of seminal vesicles*

Pt No.	Pretreatment Area (cm^2)		Posttreatment Area (cm^2)		Days
	Ipsilat	Contralat	Ipsilat	Contralat	Before + After Treatment
1	3.09	2.16	2.28	0.91	21
2	6.80	3.63	3.09	3.57	21
3	2.85	1.74	2.03	2.11	19
4	3.08	2.77	No data available	No data available	No data available
5	2.60	1.36	2.35	1.68	6
6	1.58	1.36	No data available	No data available	No data available
7	3.32	2.34	2.10	2.23	24
8	3.03	2.98	2.14	2.49	7
9	2.59	3.02	1.95	2.24	96
10	2.46	2.07	1.46	1.75	23
11	4.75	2.81	2.81	1.58	63
12	3.04	2.33	2.33	2.04	81
13	2.41	2.92	2.07	2.01	19
Totals	Mean ± SD 3.2 ± 1.3	Mean ± SD 2.4 ± 0.7	Mean ± SD 2.2 ± 0.4	Mean ± SD 2.1 ± 0.5	Median 24

Patients 1 and 8 were excluded from statistical analysis because of *no* available data after treatment.
Pretreatment ipsilateral vs contralateral and vs posttreatment ipsilateral, and pretreatment vs posttreatment contralateral paired t test p = 0.015, 0.003 and 0.053, respectively.

not detected in any of the 13 patients. *Escherichia coli, Streptococcus agalactiae* or *Flavobacterium indologenes* was isolated from the urine of 4 of the 13 patients. Patients 9 to 13 were 40 years old and older. Only 1 patient showed positive isolation of the same bacterium, *E. coli*, from urine and from fluid of the seminal vesicle on the side ipsilateral to epididymitis. One patient in the study did not have any microorganisms, probably because of antimicrobial treatment given elsewhere before his visit. No patients had any microorganism in fluid from the seminal vesicle on the contralateral side. Eradication of microorganisms was confirmed by urine examination after antimicrobial treatment in all 13 patients.

DISCUSSION

The results of our study clearly indicate that seminal vesiculitis is a discrete disease entity associated with acute epididymitis. The first evidence to support our interpretations was that abnormal dilatation of the seminal vesicle was clearly found more frequently on the side ipsilateral to epididymitis than on the contralateral side (92% vs 31%). Also, puncture fluid from the seminal vesicle on the ipsilateral side in all patients contained many white blood cells, indicating inflammation. In addition, the frequency of inflammatory findings was significantly higher in fluid from the ipsilateral seminal vesicle than from the contralateral one (100% vs 40%) when the puncture was successfully accomplished. As discussed, clinically significant microorganisms were detected in the fluid of more than 60% of patients. When these microorganisms were detected in the fluid, they were identical to those detected in urine. Furthermore, the reduction in the ipsilateral seminal vesicle paralleled the clinical outcome after appropriate antimicrobial treatment, also indicating the inflammatory nature of the infectious process. Thus, our results not only confirm the findings of previous studies,[1-3] but also add direct evidence that the seminal vesicle was strongly involved in the inflammation process.

It was intriguing that in our study *C. trachomatis* was detected in seminal vesicle fluid in younger patients with acute epididymitis, in whom the same organism was also detected in urine. Berger et al reported that *C. trachomatis* was isolated from the urethra and puncture fluid of epididymis in young patients with acute epididymitis.[5] It is well-known evidence that *C. trachomatis* can cause epididymitis. To our knowledge the results of our study provide for the first time evidence that *C. trachomatis* is involved in the development of seminal vesiculitis as well as epididymitis. In fact, in heterosexual men younger than 35 years bacteriuria is uncommon, whereas urethritis caused by *N. gonorrhoeae* or *C. trachomatis* is common.[6]

The last and most important question is whether seminal vesiculitis precedes acute epididymitis. In our study several patients with epididymitis already had dilatation and inflammation of the seminal vesicle on the contralateral side despite the finding that epididymitis was unilaterally limited, although the frequency of this abnormality was clearly lower than that on the ipsilateral side. Krishnan and Heal postulated in their study that epididymitis originated from seminal vesiculitis but the opposite did not occur.[3] If seminal vesiculitis precedes acute epididymitis, the seminal vesicle might be an infectious site of microorganisms, especially in the case of *C. trachomatis* infection. If this interpretation is correct, what is the origin of seminal vesiculitis? In the 1930s Hyams et al had already postulated 3 ways by which the disease might develop, namely direct extension from the posterior urethra with the highest probability, followed by extension from tuberculous epididymitis and blood-borne dissemination.[7] Since we have direct evidence that *C. trachomatis* is the causative microorganism of seminal vesiculitis, it is valid to speculate that the microorganism induced urethral infection caused seminal vesiculitis to develop, followed by epididymitis in some patients. However, we are still unable to explain why not all patients with chlamydial urethritis have seminal vesiculitis and epididymitis. Additional study is needed to establish the time courses.

A limitation of our study is that it is technically difficult to puncture seminal vesicles without dilatation. In this series 8 of the 13 patients could not undergo puncture successfully. Thus, we could not completely obtain inflammatory status results on the side opposite epididymitis. In addition, this puncture technique cannot be used as a routine diagnostic procedure because it is somewhat invasive for patients and the treatment for seminal vesiculitis would be the same as that for acute epididymitis.

CONCLUSIONS

Imaging, cytological and microbiological studies revealed a clear association of seminal vesiculitis with acute epididymitis. Dilatation of the seminal vesicle with inflammatory findings on the side ipsilateral to epididymitis was frequently found in patients with acute epididymitis. *C. trachomatis* was most frequently detected in fluid of dilated seminal vesicles, especially from patients 40 years and younger. The associated abnormal dilatation of the seminal vesicle disappeared in parallel with improvement in symptoms and signs

of acute epididymitis after treatment with an appropriate antimicrobial agent. This study suggests that seminal vesiculitis can be regarded as a discrete disease entity.

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ORIGINAL ARTICLE

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Incidence of sexually transmitted diseases in Hokkaido, Japan, 1998 to 2001

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Abstract The objective of this study was to provide precise data on the incidence of sexually transmitted diseases (STDs) in Hokkaido. The goal of this prospective surveillance, study was to clarify the STD incidence between 1998 and 2001 in Hokkaido, Japan. The incidence of gonococcal infection in men was found to be 127–199 per 100000 people per year, which was three or four times higher than that for women. Female genital chlamydial infection had an incidence of 300–400 with a female to male ratio of two or three to one. Younger adults had higher incidences of gonococcal and chlamydial infections than older people. In conclusion, the current study of STDs revealed high incidences of gonococcal and chlamydial infections in the Hokkaido area, and there was no decreasing trend in STD incidence during these 4 years.

Key words Sexually transmitted diseases · Surveillance · Hokkaido

Introduction

Chlamydia trachomatis and *Neisseria gonorrhoeae* are commonly prevalent in Japan. While there have been a few reports in Japan of *C. trachomatis* resistant to antimicrobial agents, many studies have indicated an increase of *N. gonorrhoeae* resistant to the conventional agents, especially to quinolone.[1] Thus, information on the incidence of sexually transmitted diseases (STDs) must be delivered to the public to establish effective countermeasures against the diseases. Unfortunately, until now, there have been no sources of data in Japan to determine the current incidences of STDs.

In this context, the Selected Prefectures Survey for STDs started in 1998 in eight prefectures of Japan with the support of Health and Labor Sciences Research Grants (Research on Emerging and Re-emerging Infectious Diseases) from the Ministry of Health, Labor, and Welfare of Japan.[2–5] The results of the studies in all selected prefectures will be reported separately. We are actively engaged in the study and responsible for data collection in Hokkaido which is the northern main island of Japan. We determined in this study the incidence of STDs in Hokkaido from 1998 through 2001.

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Patients and methods

Subjects and data collection

Hokkaido has a population of 5700000, and approximately 1800000 people live in Sapporo, the capital. The study consisted of collecting the age and sex of all newly diagnosed symptomatic patients with STDs, including syphilis; chancroid; genital herpes infection; condyloma acuminatum; and gonococcal, chlamydial, and nongonococcal and nonchlamydial infections of the urethra or uterine cervix, in June and November in 1998, 1999, 2000, and 2001. The data were requested from all clinics and hospitals that were engaged in the treatment of patients with STDs. By mail, we asked all these clinics and hospitals in Hokkaido to participate in the study and report these data.

Diagnosis of STDs

The early stage of symptomatic syphilis was diagnosed by skin manifestation and standard serum tests. Chancroid, genital herpes infection, and condyloma acuminatum were

basically diagnosed through inspection by physicians to identify typical clinical lesions. Symptomatic patients with urethritis or cervicitis were diagnosed as having gonococcal infection when *N. gonorrhoeae* was detected in urethral discharge or the first voided urine in male patients and cervical smears in female patients. The detection methods for this organism depended on the clinic and included Gram staining, culture, polymerase chain reaction (PCR), and ligase chain reaction (LCR). Symptomatic patients with *C. trachomatis* infection were diagnosed as having the infection by enzyme-linked immunoassay, PCR, or LCR methods in specimens similar to those used in gonococcal detection. When neither *C. trachomatis* or *N. gonorrhoeae* was detected in symptomatic patients, they were diagnosed as having nonchlamydial and nongonococcal (NC-NG) infection of the urethra or cervix. If examination to detect *C. trachomatis* was not done and patients showed no typical findings of gonococcal infection, they were diagnosed as having nongonococcal infection with chlamydia not determined (NG-CND) of the urethra or cervix.

Estimation of incidence of STDs

The incidence of STDs was determined as the number of patients per 100000 people per year, based on the results of the two months (June and November) and the total population of Hokkaido in the corresponding year. The final incidence was adjusted by the response rates of institutes in a given year.

Results

During the 4 years of the study, the number of institutes asked to report information about patients with STDs varied from 578 to 711 as a result of the opening of new opened hospitals and the closure of old ones (Table 1). However, response rates were consistently high at around 80%, suggesting that most of the clinics and hospitals actively participated in the study.

When all STDs were taken into consideration, the mean incidences from 1998 through 2001 were 590 male patients and 816 female patients per 100000 people per year (Table 2). Although classic STDs such as syphilis and chancroid showed very low incidences, the rates of gonococcal and chlamydial infections in male patients and chlamydial infection in female patients were high in Hokkaido. In particular, the incidence of chlamydial infection in female patients was

Table 1. Number of institutes asked to participate and response rates in June and November each year

	No. of institutes	Response rate (%)
1998		
June	584	82.2
November	578	80.3
1999		
June	711	78.9
November	697	84.1
2000		
June	683	78.6
November	679	79.2
2001		
June	656	87.8
November	644	87.1
Mean		82.3

Table 2. Incidence (per 100000 people per year) of sexually transmitted diseases (STDs) from 1998 through 2001 in Hokkaido prefecture

STD	1998		1999		2000		2001	
	Male	Female	Male	Female	Male	Female	Male	Female
All STDs	444 (436.0–453.7)	688 (677.5–698.7)	630 (619.6–640.4)	910 (897.7–921.7)	621 (610.2–631.2)	853 (840.9–864.6)	663 (652.2–672.9)	813 (801.7–823.6)
Syphilis	1.4 (0.9–1.9)	1.3 (0.8–1.8)	1.9 (1.3–2.5)	1.5 (1.0–2.0)	2.5 (1.9–3.2)	5 (0.2–0.8)	1.0 (0.6–1.4)	0.7 (0.4–1.0)
Chancroid	0	0	0	0	0	0	0	0.2 (0.1–0.4)
Genital herpes infection	31 (29.1–33.8)	67 (63.4–70.0)	33 (30.6–35.4)	64 (61.2–67.6)	35 (32.4–37.4)	71 (68.0–74.9)	33 (30.2–34.8)	65 (61.6–67.8)
Condyloma acuminatum	20 (17.9–21.6)	26 (24.0–28.1)	26 (23.5–27.7)	33 (31.0–35.6)	28 (25.9–30.4)	35 (32.8–37.6)	23 (21.0–24.8)	29 (26.7–30.8)
Gonococcal infection	127 (121.9–131.3)	30 (27.7–32.1)	190 (184.4–195.8)	51 (48.7–54.4)	162 (156.8–167.6)	53 (50.5–56.4)	199 (193.4–204.9)	62 (59.1–65.2)
Chlamydial infection	100 (96.2–104.6)	273 (266.1–279.5)	146 (141.4–151.5)	378 (370.3–385.8)	156 (150.7–161.3)	341 (333.6–348.6)	178 (172.8–183.5)	353 (345.5–360.0)
NC-NG infection	139 (134.4–144.3)	240 (233.9–246.3)	197 (191.3–203.0)	316 (308.6–322.8)	210 (203.7–215.9)	313 (305.8–320.1)	217 (211.1–222.9)	281 (274.5–287.5)
CND-NG infection	26 (23.7–28.0)	51 (48.4–54.2)	36 (33.3–38.2)	41 (38.4–43.5)	26 (24.3–28.6)	16 (15.9–14.3)	11 (10.1–12.8)	5 (4.1–5.8)

Data are median incidence values with the 95% confidence interval in parentheses
NC-NG, nonchlamydial and nongonococcal infection; CND-NG, nongonococcal infection with chlamydia not determined

two to three times higher than in male patients. This finding was consistent throughout the 4 years. In contrast, gonococcal infection was predominantly found in male patients.

Gonococcal infection (Table 3) and genital chlamydial infection (Table 4) were high in younger people. The peak incidence was found at the ages of 20–24 years for both infections, followed by 15–19 and 25–29. In younger people, there was a much higher incidence of female chlamydial infection than male gonococcal infection.

Discussion

Countermeasures to prevent the spread of STDs have been a major medical and public health issue. Some countries have been very active in the prevention of STDs because prevention is closely linked with a decrease in the incidence of HIV infection.[6,7] Thus, it is crucial as a first step in the prevention of STDs to understand current trends in STD incidence. Unfortunately, we have not had appropriate sources of data in Japan to estimate the incidence of STDs. The major purpose of this study is to provide such data and to estimate the incidences of STDs.[2–5,8]

Hokkaido is located in northern Japan and is the largest prefecture in Japan. It is rich in natural resources and has many tourist attractions and many tourists from not only other cities in Japan but also other Asian countries. Sapporo is the capital of Hokkaido with a population of approximately 2 million. Sapporo's Susukino entertainment district has been thought to be a major source of STDs. In addition, the style of sexual activity has been changing slowly since the late 1990s, with oral sex becoming more common. We have already investigated differences in STD incidence between urban Sapporo and the rural areas of Hokkaido.[8] The levels of chlamydial infection were almost the same in urban and rural areas; however, the incidence of gonococcal infection in male patients was higher in urban areas than in rural areas.

The Centers for Disease Control and Prevention (CDC), USA, reported the overall rates of chlamydial infection in the USA in 1995 to be 290.3 in women and 52.1 in men per 100000 population.[9] In the CDC report, the incidence had declined substantially for all age groups over a seven-year period, although they were persistently highest among young adolescents. In Birmingham, UK, the overall prevalence of chlamydia was reported to be 129 per 100000.[10] Northern Australia had a reported incidence of female chlamydial infection of 250 per 100000.[11] The rate for women was approximately six times higher than that for men. In addition, the report indicated that those aged 15–19 years accounted for 46% of those infected, followed 20–24 years at 33% and 14 years and younger at 4%. Thus, the report cautioned that a higher incidence of the infection was evident in the younger generation. Similar findings were apparent in our study. Moreover, the incidence of chlamydial infection in the study was higher than those reported in other countries. Simple comparison of the incidence levels

Table 3. Incidence (per 100000 people per year) of gonococcal infections according to age category

Age (years)	Gonococcal infection (male)				Gonococcal infection (female)			
	1998	1999	2000	2001	1998	1999	2000	2001
10–14	0	4 (0.9–7.6)	0	8 (3.5–12.3)	0	13 (7.2–19.4)	23 (14.7–31.0)	0
15–19	166 (146.2–186.0)	252 (227.4–275.8)	241 (216.6–264.8)	245 (221.7–267.9)	170 (139.2–190.3)	224 (201.0–247.9)	232 (207.6–256.0)	227 (204.5–249.9)
20–24	513 (478.6–547.4)	715 (674.6–755.1)	579 (541.9–615.5)	613 (576.6–648.6)	165 (145.4–184.5)	309 (282.6–335.6)	263 (238.6–288.3)	312 (285.8–337.1)
25–29	401 (368.3–435.3)	749 (703.5–793.9)	545 (506.6–584.4)	875 (827.9–922.3)	41 (30.5–50.9)	100 (83.9–115.6)	185 (163.5–207.4)	205 (182.6–226.4)
30–34	325 (295.2–355.3)	517 (480.0–554.5)	414 (379.7–447.9)	522 (485.6–558.3)	24 (20.0–37.1)	60 (47.7–72.3)	54 (41.9–65.5)	93 (78.4–108.0)
35–39	172 (150.4–193.6)	219 (195.0–243.3)	252 (226.1–278.8)	295 (267.6–321.6)	21 (13.2–27.9)	36 (26.7–45.9)	21 (13.4–28.2)	49 (38.1–59.6)
40–44	89 (74.8–103.1)	129 (112.2–145.9)	133 (115.8–150.7)	172 (153.4–191.0)	0	10 (5.3–14.4)	14 (8.2–19.0)	21 (15.0–27.9)
45–49	65 (53.1–76.1)	73 (60.8–84.9)	56 (44.9–66.3)	80 (67.5–91.9)	0	6 (2.6–9.1)	9 (4.9–13.2)	5 (2.4–8.5)
50–54	53 (41.6–65.1)	68 (55.2–81.6)	71 (57.0–84.3)	34 (24.8–42.7)	7 (3.2–11.4)	0	4 (0.8–6.7)	13 (8.1–18.7)
55–59	9 (3.8–13.6)	17 (10.3–23.9)	13 (7.2–19.3)	10 (12.9–27.0)	8 (3.4–12.2)	0	0	7 (3.1–11.2)
60–64	13 (7.2–19.3)	9 (3.8–13.5)	22 (14.4–30.3)	40 (30.2–50.4)	4 (0.9–7.4)	8 (3.6–12.7)	0	0
65+	4 (1.9–6.6)	2 (0.4–3.7)	11 (6.9–14.6)	4 (1.7–6.1)	2 (0.3–2.8)	2 (0.3–2.8)	0	0

Table 4. Incidence (per 100000 people per year) of chlamydial infections according to age category

Age (years)	Chlamydial infection (male)				Chlamydial infection (female)			
	1998	1999	2000	2001	1998	1999	2000	2001
10–14	0	4 (0.9–7.6)	0	8 (3.5–12.3)	9 (4.0–14.1)	31 (21.7–40.3)	23 (14.7–31.0)	12 (6.7–18.1)
15–19	185 (163.9–205.9)	377 (347.7–407.1)	290 (263.9–316.8)	303 (277.7–329.1)	1255 (1199.4–1311.2)	1540 (1479.0–1601.6)	1547 (1484.1–1608.9)	1439 (1381.7–1496.1)
20–24	451 (418.4–483.0)	665 (625.8–703.4)	664 (624.6–703.4)	723 (684.0–762.1)	1481 (1422.5–1539.5)	2286 (2213.8–2357.6)	1893 (1826.1–1959.1)	1892 (1828.9–1955.3)
25–29	269 (241.9–296.7)	381 (348.6–413.1)	608 (566.3–649.1)	653 (612.5–694.1)	757 (713.0–801.2)	1125 (1072.1–1178.6)	940 (890.1–988.9)	1253 (1199.0–1307.4)
30–34	277 (249.2–304.6)	293 (264.8–321.2)	285 (256.5–313.0)	381 (350.3–412.5)	326 (297.4–355.4)	380 (349.0–410.9)	405 (372.3–437.2)	432 (400.4–464.2)
35–39	103 (86.5–119.9)	156 (135.6–176.3)	192 (168.6–214.5)	208 (185.5–230.9)	115 (97.8–132.3)	153 (133.4–172.8)	175 (153.3–196.1)	195 (173.7–216.7)
40–44	68 (55.3–79.9)	84 (70.1–97.3)	79 (65.8–92.7)	120 (104.5–136.0)	40 (31.0–49.5)	49 (39.2–59.4)	68 (55.8–79.9)	31 (22.9–38.3)
45–49	32 (24.2–40.4)	51 (40.6–60.7)	43 (33.2–51.9)	71 (59.3–82.3)	24 (17.2–30.6)	29 (21.9–36.6)	18 (12.3–24.0)	22 (15.7–27.9)
50–54	29 (20.1–37.4)	28 (19.7–36.6)	54 (42.1–65.9)	49 (38.0–59.5)	11 (5.9–16.0)	29 (20.7–36.8)	26 (18.2–33.7)	17 (10.8–22.7)
55–59	13 (7.1–19.1)	30 (21.0–38.9)	18 (10.7–24.7)	24 (16.2–31.7)	16 (9.5–21.9)	12 (6.6–16.8)	4 (0.8–7.1)	7 (3.1–11.2)
60–64	4 (0.9–7.9)	4 (0.9–7.8)	9 (3.9–14.0)	12 (6.5–17.6)	12 (6.7–18.1)	12 (6.6–17.7)	8 (3.7–13.1)	8 (3.3–11.8)
65+	2 (0.4–3.8)	2 (0.4–3.7)	2 (0.4–3.9)	4 (1.7–6.1)	2 (0.3–2.8)	3 (1.4–4.8)	3 (1.4–5.0)	0

sometimes is not appropriate because of different backgrounds of study design. However, the results of our study suggest that we need to provide immediately effective countermeasures to prevent chlamydial infection in the younger generation.

In our study, the incidence of male gonococcal infection was 127–199 per 100000 people per year and the incidence for women was 30–62. In other reports, the overall incidence of male gonococcal infection was 98.4 per 100000 men and the estimated incidence of female gonococcal infection was 370 per 100000.[11] In the USA, the incidence of gonococcal infection declined 71.3% between 1981 and 1996.[12] However, there were some regions still having high rates of infection, such as 547.4 per 100000 in Kansas City, MO, 669.7 in Detroit, MI, 939.8 in Baltimore, MD, and 942.5 in Newark, NJ. Interestingly, there were some states, such as Montana (4.4 per 100000) and North Dakota[9,12] with low infection rates. In Hokkaido, there was no declining trend of the disease during the 4 years of the study. The incidence of gonococcal infection in men was three to four times higher than that in women, and the trend is clearly different from that of chlamydial infection. We still cannot explain exactly why the incidence of male gonococcal infection is higher than that of female infection. The traditional explanation of the mild nature of the infection in women may be valid, because some screening programs for high-risk groups have a nonnegligible detection rate of gonococcal infection.[13,14] Screening programs for gonococcal infection may reveal higher incidences of the infection. In our study, only symptomatic patients were asked to be reported, so that nonsymptomatic patients with the infection would have been excluded from the data provided by institutes.

The incidence of genital herpes infection and condyloma acuminatum were low in this study. The incidence of latent or subclinical infections with herpes simplex virus (HSV) and human papillomavirus (HPV) has been reported to be higher than that of symptomatic infection.[15,16] Indeed, we already reported that HPV was detected in healthy men and men with urethritis. In particular, 18% of those with urethritis had HPV DNA on their external genitalia.[17] It is noteworthy that more than 80% of positive patients had high- or intermediate-oncogenic-risk HPV DNA. However, the detection seems to be transient so that symptomatic infection may be less prevalent, as found in our study.

Our study had several limitations in design. Although there were consistently high response rates throughout the 4 years of the study, variation in the institutes that participated may have affected the number of patients with STDs being reported, so that the total numbers might vary somewhat. The second is that diagnostic procedures for STDs, in particular for gonococcal or chlamydial infections, might not be the same in different clinics and hospitals. Some nongonococcal infections with chlamydia not determined may have been chlamydial infections because a specific detection test for chlamydia was not done. Furthermore, some detection procedures such as Gram staining for gonococcus have a definitely lower sensitivity than PCR or LCR. Each

institute that participated in the study had its own detection policy for the causative agent of STDs. This might also have affected the results. Nevertheless, the study is the first comprehensive one that allows us to estimate much more precisely the incidence of STDs. Thus, we now clearly understand that the incidence of STDs in our prefecture is higher than previously anticipated, in particular, those of gonococcal and chlamydial infections in younger people. These results emphasize the need to establish effective countermeasures for prevention, such as robust health education about STDs.

In conclusion, we conducted a prefecture-wide survey for STDs. High response rates for reporting the number of patients from each institute enabled us to estimate the incidence of STDs in Hokkaido. Incidences of gonococcal and chlamydial infections were prominently high, especially in younger people. The results clearly indicate the need for prompt establishment of practical countermeasures for prevention of these diseases.

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Antimicrobial resistance of *Neisseria gonorrhoeae* in Japan, 1993–2002: continuous increasing of ciprofloxacin-resistant isolates

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Antimicrobial resistance of *Neisseria gonorrhoeae* in Japan, 1993–2002: continuous increasing of ciprofloxacin-resistant isolates

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Abstract

Susceptibility testing was conducted on 1357 isolates of *Neisseria gonorrhoeae* isolated from 1993 through 2002 in Japan to assess the antimicrobial resistance. Selected isolates were characterised by auxotype and analysis was done for mutations within the quinolone resistance-determining region (QRDR) in the *gyrA* and *parC* genes, which confer fluoroquinolone resistance to the organism. Isolates with ciprofloxacin resistance increased significantly from 6.6% (1993–1994) to 73.5% (2002). The proportion of plasmid-mediated penicillin-resistant isolates (PPNG) decreased significantly from 7.9% (1993–1994) to 0.9% (2002). The percentage of chromosomal-mediated resistance to penicillin decreased from 27.4% in 2000 to 12.0% in 2001 but increased to 28.9% in 2002. The proportion of isolates with any type of resistance to tetracycline decreased from 24.7% in 2000 to 13.9% in 2001 and then increased to 22.3% in 2002. The proportion of prototrophic isolates significantly decreased from 84.4% in 1992–1993 to 7.7% in 2001, while that of the proline-requiring isolates significantly increased from 4.4% in 1992–1993 and 80.8% in 1998. The proline-requiring isolates were less susceptible to ciprofloxacin than the prototrophic or arginine-requiring isolates. Of 87 isolates resistant to ciprofloxacin, 2 (2.3%) contained five amino acid substitutions within the GyrA and ParC proteins, 76 (87.4%) contained three or four amino acid substitutions and 9 (10.3%) contained one or two amino acid substitutions.

Keywords: *Neisseria gonorrhoeae*; Resistance; Auxotype; DNA gyrase; Topoisomerase IV

1. Introduction

The incidence of gonococcal infection has been increasing since the mid-1990s in Japan. Gonococcal resistance to antimicrobial agents has also been an increasing problem in the treatment of gonorrhoea in Japan. In the last few years, a high prevalence of the fluoroquinolone-resistant *Neisseria gonorrhoeae* isolates and the treatment failure of gonococcal infections with fluoroquinolones have been recognised in Japan [1,2]. Presently, *N. gonorrhoeae* isolates have evolved in acquiring mutidrug resistance to fluoroquinolone, penicillin and tetracycline. Auxotyping involves the characterisation of nutritional requirements for the growth of *N. gonorrhoeae* isolates. Our previous investigation demonstrated that the proline-requiring isolates of *N. gonorrhoeae* were less susceptible to ciprofloxacin than either prototrophic isolates or arginine-requiring isolates [1].

This study was performed to characterise the current antimicrobial susceptibility of *N. gonorrhoeae* and in particular, to examine the possibility of emerging high prevalence of high-level fluoroquinolone resistance and multidrug resistance in Japan. In addition, we examined the changes of gonococcal auxotype and amino acid substitutions in DNA gyrase subunit A (GyrA) and topoisomerase IV *parC*-encoded subunit (ParC) proteins, which confer quinolone resistance to the organisms.

2. Materials and methods

2.1. *Neisseria gonorrhoeae* strains

From January 1993 through December 2002, a total of 1357 isolates of *N. gonorrhoeae* (1993–1994: 151 isolates; 1995–1996: 154 isolates; 1997–1998: 197 isolates; 1999: 246 isolates; 2000: 190 isolates; 2001: 208 isolates; and 2002: 211 isolates) were collected from consecutive male

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patients with urethritis attending a sexually transmitted diseases (STD) clinic in Fukuoka city, Japan. Post-treatment isolates or repeat isolates from the same patients were excluded. Specimens from each patient were inoculated directly onto Thayer–Martin selective agar (Becton Dickinson, Cockeysville, MD, USA), transported to the Mitsubishi Kagaku Laboratory and incubated for 24–48 h at 35 °C in 5% CO_2 atmosphere. *N. gonorrhoeae* was identified as Gram-negative diplococci and by oxidase reaction and sugar utilisation patterns. The isolates were stored at −80 °C until they were tested.

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) for all isolates were determined by an agar dilution technique with a GC agar base (Becton Dickinson) containing 1% Iso VitaleX (Becton Dickinson) and two-fold dilutions of antibiotic as described previously [1]. The plates were inoculated with approximately 10^4 colony-forming units (cfu) per spot of each isolate with a multipoint inoculator for a brief period. World Health Organization (WHO) reference *N. gonorrhoeae* strains A, B, C, D and E, and *N. gonorrhoeae* ATCC49226 strain were included as quality controls. The plates were incubated for 24 h at 35 °C in 5% CO_2 atmosphere. MICs were defined as the lowest antibiotic concentration observed to inhibit bacterial growth. β-Lactamase production was assayed using the chromogenic cephalosporin test (Nitrocefin, Oxoid, Hampshire, UK). The antimicrobial agents tested were penicillin G (Sigma Chemical St. Louis, MO, USA), tetracycline (Wyeth Ledere Japan, Tokyo, Japan), cefixime (Fujisawa Pharmaceutical, Osaka, Japan), ceftriaxone (Nippon Roche, Tokyo, Japan), ciprofloxacin (Bayer Yakuhin, Osaka, Japan), levofloxacin (Daiichi Pharmaceutical, Tokyo, Japan), sitafloxacin (Daiichi Pharmaceutical, Tokyo, Japan), gatifloxacin (Kyorin Pharmaceutical, Tokyo, Japan), azithromycin (Pfizer Pharmaceuticals, Tokyo, Japan) and spectinomycin (Sigma Chemical). All the antibiotics were obtained in powder-form with the stated potencies of their manufacturers. The antimicrobial susceptibility was judged by breakpoint criteria defined by the National Committee for Laboratory Standards (NCCLS) [3].

2.3. Auxotyping

Auxotyping of gonococcal isolates was performed as described by Catlin [4]. The isolates were tested on the chemically defined media for their nutritional requirements for proline, arginine, hypoxanthine, uracil, lysine, leucine, methionine, histidine and combinations of these requirements. Argine-requiring isolates were also cultured on media for determining their ability to utilise ornithine as an alternative substrate. The strains with no requirements for these substances were designated as prototrophic.

2.4. Molecular study

To identify mutations in the *gyrA* and *parC* genes of the 89 gonococcal strains (1992–1998 isolates) and 223 gonococcal strains (1999 isolates), the polymerase chain reaction (PCR) and direct DNA sequencing were performed as described previously [1]. To amplify the genes corresponding to the quinolone resistance-determining region (QRDR) within the GyrA and ParC proteins, the oligonucleotide primers for the PCR amplification were designed [1].

2.5. Statistical analysis

Data were analysed by chi-square test. Statistical significance for all P-values was set at 0.05.

3. Results and discussion

3.1. Fluoroquinolone resistance

The proportion of isolates resistant to ciprofloxacin (MIC $\geq$ 1 mg/l) increased remarkably from 6.6% in 1993–1994 to 73.5% in 2002 (Fig. 1). This difference was statistically significant ($P < 0.0001$). The ciprofloxacin MIC_{50} (4 mg/l) and MIC_{90} (32 mg/l) for isolates in 2002 were 128- and 64-fold, respectively, which were higher than those (MIC_{50}: 0.03 mg/l and MIC_{90}: 0.5 mg/l) for the isolates in 1993–1994. The levofloxacin MIC_{50} (4 mg/l) and MIC_{90} (8 mg/l) for the isolates in 2002 were also 128- and 32-fold, respectively, which were higher than those (MIC_{50}: 0.03 mg/l and MIC_{90}: 0.25 mg/l) for the isolates in 1993–1994. Moreover, gonococcal isolates in 2002 showed resistance to a new fluoroquinolones of sitafloxacin and gatifloxacin (Table 1). In Fukuoka city, about 75% of *N. gonorrhoeae* isolates have been found to be resistant to ciprofloxacin. These findings seem to reflect the longstanding usage of fluoroquinolones as a drug of choice for the treatment of gonococcal infection at sexually transmitted disease clinics in Fukuoka city. The frequent use of fluoroquinolones against gonorrhoea may lead to the rapid development of resistance to these agents. This increase in fluoroquinolone resistance has also been substantial in some Western Pacific countries. High proportions of fluoroquinolone-resistant *N. gonorrhoeae* are detected in China (85.2%), Hong Kong (79.5%), the Philippines (37.9%) and Vietnam (42.7%). The percentage of fluoroquinolone-resistant isolates in Hong Kong has increased from about 50% in 1998 to 79.5% in 2000 and the fluoroquinolone resistance rates have increased markedly in China [5].

3.2. Penicillin resistance

The proportion of isolates with any type of resistance to penicillin (MIC $\geq$ 2 mg/l) decreased from 27.9% in 2000 to

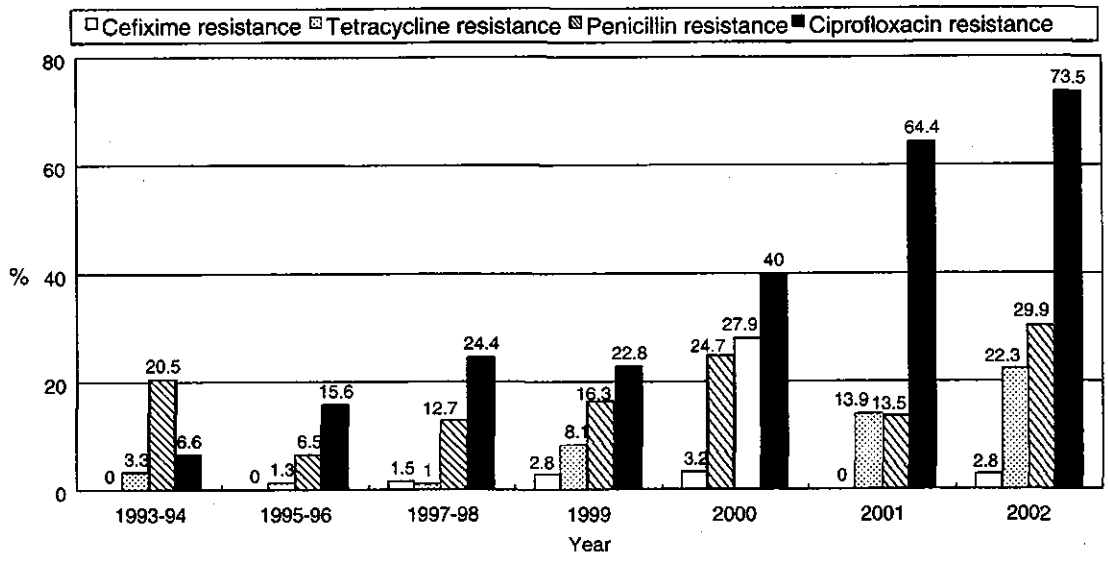


Fig. 1. Changes in the proportion of the various antimicrobial-resistant *N. gonorrhoeae* isolates.

Table 1
Susceptibilities of *N. gonorrhoeae* isolates to fluoroquinolones and penicillin

Antibiotic	Year	MIC (mg/l)		
		50%	90%	Range
Ciprofloxacin	1993–1994	0.03	0.5	≤0.001–1
	1995–1996	0.03 (1×)	1 (2×)	0.004–16
	1997–1998	0.03 (1×)	8 (16×)	0.002–16
	1999	0.12 (4×)	8 (16×)	≤0.001–16
	2000	0.25 (8×)	16 (32×)	≤0.001–64
	2001	4 (128×)	16 (32×)	≤0.001–64
	2002	4 (128×)	32 (64×)	0.002–64
Levofloxacin	1993–1994	0.03	0.25	≤0.001–0.5
	1995–1996	0.06 (2×)	1 (4×)	0.008–8
	1997–1998	0.06 (2×)	8 (32×)	0.004–16
	1999	0.25 (8×)	4 (16×)	≤0.001–16
	2000	0.5 (16×)	8 (32×)	0.002–32
	2001	2 (64×)	8 (32×)	0.002–16
	2002	4 (128×)	8 (32×)	0.004–32
Sitafloxacin	1993–1994	0.004 (1×)	0.015 (1×)	0.001–0.03
	1995–1996	0.004 (2×)	0.03 (2×)	≤0.001–0.25
	1997–1998	0.008 (2×)	0.12 (8×)	≤0.001–0.5
	2001	0.12 (32×)	0.25 (16×)	≤0.001–0.25
	2002	0.12 (32×)	0.25 (16×)	≤0.001–0.5
Gatifloxacin	1993–1994	0.015 (2×)	0.06 (1×)	≤0.001–0.12
	1995–1996	0.03 (2×)	0.25 (4×)	0.004–2
	2001	1 (64×)	2 (32×)	≤0.001–4
	2002	1 (64×)	2 (32×)	≤0.001–8
PenicillinG[a]	1993–1994	0.25	2	0.008–2
	1995–1996	0.12 (0.5×)	1 (0.5×)	0.015–2
	1997–1998	0.25 (1×)	2 (1×)	0.03–4
	1999	0.25 (1×)	2 (1×)	0.008–4
	2000	0.5 (2×)	2 (1×)	0.008–8
	2001	0.5 (2×)	2 (1×)	0.004–4
	2002	1 (4×)	2 (1×)	0.004–4

[a] Non-PPNG isolates only.

13.5% in 2001 but increased to 29.9% in 2002 (Fig. 1). The prevalence of plasmid-mediated penicillin-resistant gonococci (PPNG) strains decreased significantly from 7.9% in 1993–1994 to 0.9% in 2002 ($P < 0.002$). However, the percentage of chromosomal-mediated resistance (CMRNG) to penicillin decreased from 27.4% in 2000 to 12.0% in 2001 and then increased to 28.9% in 2002. The MIC_{50} of penicillin for non-PPNG in 2002 were four-fold higher than those in 1993–1994. However, the MIC_{90} of penicillin for non-PPNG in 1997–1998 were similar to that in 1993–1994 (Table 1). High prevalence of penicillin-resistant gonococcal isolates remains a major problem in many parts of the Western Pacific countries. Very high rates of combined forms of penicillin resistance (CMRNG + PPNG) are recorded in Korea (91%), the Philippines (89%), China (80%), Brunei (63%), Singapore (58%), Hong Kong (54%) and Vietnam (48%) [5]. The prevalence rates of penicillin-resistance in these Western Pacific countries are much higher than that in Japan.

3.3. Tetracycline resistance

The proportion of isolates with any type of resistance to tetracycline decreased from 24.7% in 2000 to 13.9% in 2001 and then increased to 22.3% in 2002 (Fig. 1). Only two (0.15%) isolates of plasmid-mediated high-level tetracycline resistance (TRNG) were identified during the study period. The tetracycline MIC_{50} and MIC_{90} for the isolates in 2002 were only two-fold higher than those for the isolates in 1993–1994 (Table 2). High rates of TRNG (between 25 and 70%) were prominent in Malaysia, Brunei, Singapore, Vietnam, China and Papua New Guinea. The prevalence rate of TRNG in Japan is remarkably lower than those in the countries mentioned. In other Western Pacific countries rates

Table 2
Susceptibilities of *N. gonorrhoeae* isolates to various antibiotics

Antibiotic	Year	MIC (mg/l)		
		50%	90%	Range
Tetracycline	1993–1994	0.5	1	0.06–8
	1995–1996	0.25 (0.5×)	1 (1×)	0.06–4
	1997–1998	0.25 (0.5×)	2 (2×)	0.06–2
	1999	0.5 (1×)	1 (1×)	0.03–2
	2000	0.5 (1×)	2 (2×)	0.03–16
	2001	1 (2×)	2 (2×)	0.03–4
	2002	1 (2×)	2 (2×)	0.06–16
Cefixime	1993–1994	0.015	0.12	≤0.001–0.25
	1995–1996	0.008 (0.5×)	0.06 (0.5×)	≤0.001–0.12
	1997–1998	0.015 (1×)	0.12 (1×)	0.002–0.5
	1999	0.015 (1×)	0.25 (2×)	≤0.001–0.5
	2000	0.03 (2×)	0.25 (2×)	0.002–0.5
	2001	0.015 (1×)	0.25 (2×)	≤0.001–0.25
	2002	0.03 (2×)	0.25 (2×)	0.002–0.5
Ceftriaxone	1993–1994	0.015	0.06	≤0.001–0.25
	1995–1996	0.015 (1×)	0.12 (2×)	≤0.001–0.12
	1997–1998	0.008 (0.5×)	0.12 (2×)	≤0.001–0.25
	1999	0.008 (0.5×)	0.06 (1×)	≤0.001–0.12
	2000	0.015 (1×)	0.06 (1×)	≤0.001–0.5
	2001	0.015 (1×)	0.06 (1×)	≤0.001–0.06
	2002	0.015 (1×)	0.06 (1×)	0.002–0.12
Azithromycin	1993–1994	0.06	0.25	0.008–1
	1995–1996	0.06 (1×)	0.12 (0.5×)	0.015–0.5
	1997–1998	0.12 (2×)	0.25 (1×)	0.015–1
	1999	0.12 (2×)	0.5 (2×)	0.015–0.5
	2000	0.12 (2×)	0.5 (2×)	0.008–0.5
	2001	0.25 (4×)	0.5 (2×)	0.008–0.5
	2002	0.12 (2×)	0.25 (1×)	0.008–2
Spectinomycin	1993–1994	8	8	2–16
	1995–1996	8 (1×)	16 (2×)	4–16
	1997–1998	8 (1×)	16 (2×)	4–16
	1999	8 (1×)	8 (1×)	2–16
	2000	8 (1×)	16 (2×)	2–32
	2001	8 (1×)	16 (2×)	2–16
	2002	8 (1×)	16 (2×)	4–16

of TRNG range between 0.5 and 11% of strains examined [5].

3.4. Cephalosporin resistance

The proportion of isolates resistant to cefixime (MIC ≥ 0.5 mg/l) was very low, ranging from 0 to 3.2% during the study period (Fig. 1). The MIC_{50} and MIC_{90} values for the isolates in 2002 were only two-fold higher than isolates in 1993–1994. There were no significant changes in the gonococcal susceptibility to ceftriaxone. The MIC_{50} and MIC_{90} values of this agent for the isolates in the year 2002 were similar to those in 1993–1994 (Table 2). Only one isolate in the year 2000 showed reduced susceptibility to ceftriaxone (MIC = 0.5 mg/l). These data indicates that most gonococcal isolates are susceptible to ceftriaxone and cefixime. These later generation cephalosporins are very important agents in the treatment of gonorrhoea as resistance to fluoroquinolone, penicillin and tetracycline accelerates in Japan.

3.5. Spectinomycin resistance

No isolate resistant to spectinomycin (MIC ≥ 128 mg/l) was detected and there were no significant changes in the gonococcal susceptibility to spectinomycin during this period. The MIC_{50} value of spectinomycin for the isolates in the year 2002 was similar to that in 1993–1994. The MIC_{90} value for the 2002 isolates was only two-fold higher than for isolates in 1993–1994 (Table 2). A single dose of spectinomycin (2 g) is one of first-line regimens for gonorrhoea recommended by the *Japanese Society for Sexually Transmitted Diseases* and is one of the alternative regimens used in the United States [6] and the United Kingdom [7]. Our results corroborate that a single dose of spectinomycin, as one of first-line regimens, is still effective against gonococcal infection. However, only a small number of spectinomycin-resistant *N. gonorrhoeae* were found in some Western Pacific countries [5].

3.6. Azithromycin resistance

There were no significant changes in the gonococcal susceptibility to azithromycin during the study period. The MIC_{50} value of azithromycin for the isolates in 2002 was only two-fold higher than for isolates in 1993–1994. The MIC_{90} of this agent for the isolates in 2002 were equal to that in 1993–1994 (Table 2). Although azithromycin is not recommended currently for gonococcal infection in Japan, the agent could be one of the regimens to be used in gonorrhoea caused by isolates resistant to penicillin, tetracycline and/or ciprofloxacin.

3.7. Multidrug resistance

The proportion of isolates of non-β-lactamase producing *N. gonorrhoeae* (non-PPNG) resistant to any three antibiotics among penicillin, tetracycline and ciprofloxacin during three periods are shown in Fig. 2. Only five (3.1%) out of 159 non-PPNG isolates in 1997 were resistant to both penicillin and ciprofloxacin. Three types of isolates

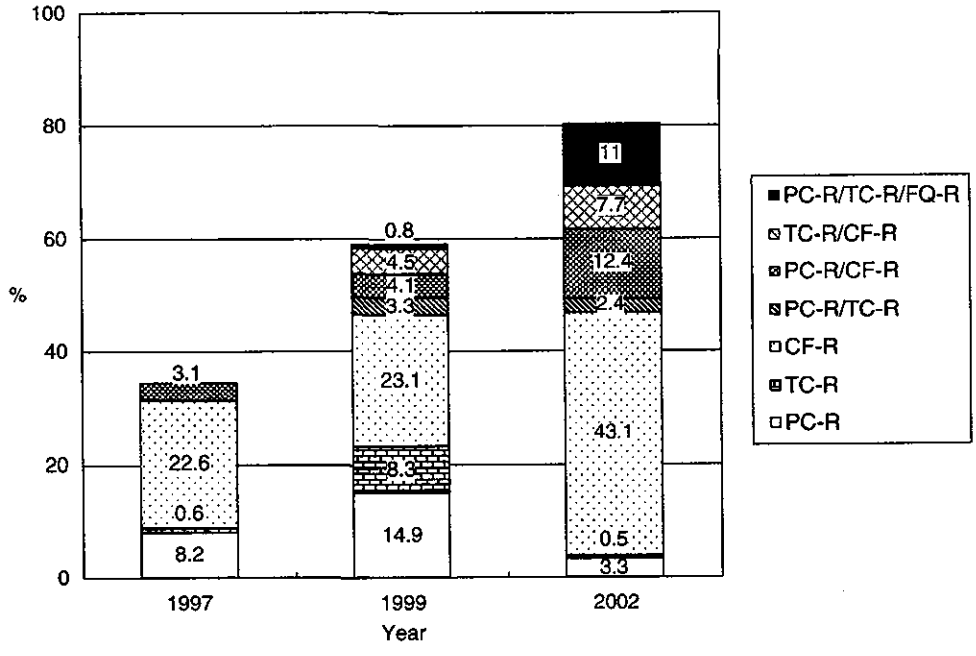


Fig. 2. Changes in the proportion of isolates of non-PPNG resistant to any three antibiotics among penicillin, tetracycline and ciprofloxacin. PC-R: penicillin resistance; TC-R: tetracycline resistance; and CF-R: ciprofloxacin resistance.

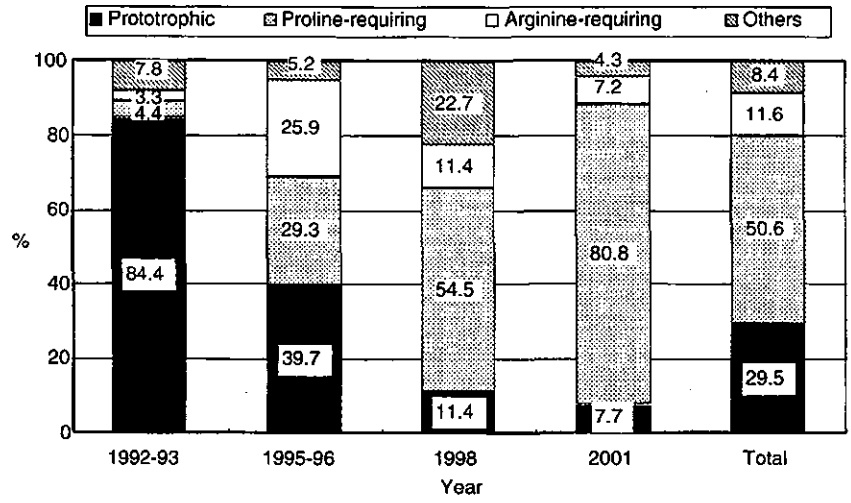


Fig. 3. Changes in the auxotypes of *N. gonorrhoeae* isolates.

resistant to two antibiotics accounted for 3.3–4.5% of the 173 non-PPNG isolates in 1999. Of the 209 non-PPNG isolates in 2002, three types of isolates resistant to two antibiotics accounted for 2.4–12.4%. Multidrug-resistant isolates (resistant to penicillin, tetracycline and ciprofloxacin) were significantly increased from 1999 (0.8%) to 2002 (11.0%) ($P = 0.0002$). These results suggest that the isolates showing resistance to ciprofloxacin have chromosomal mutations in *gyrA* with or without *parC* genes that may be combined with well-known chromosomal mutations at other loci such as *penA* (decreased binding to PBP2) [8], *penB* (reduced porin permeability) [9] and *mtr* (mutidrug efflux pump) [10]. The treatment of gonorrhoea will therefore becomes more and more complicated due to multidrug resistance to a variety of antimicrobial agents.

3.8. Auxotype

Auxotyping is a useful epidemiological marker for monitoring gonococcal epidemics. In various south-east Asian countries, both prototrophic and proline-requring isolates have been reported to be prevalent [11,12]. In this study, the three predominant auxotypes were prototrophic (29.5%), proline-requiring (50.6%) and arginine-requiring (11.6%) in a total of 502 isolates tested (Fig. 3). There were dramatic changes in the proportion of these auxotypes. The proportion of prototrophic isolates significantly decreased from 84.4% in 1992–1993 to 7.7% in 2001 ($P < 0.0001$), while that of the proline-requiring isolates significantly increased from 4.4% in 1992–1993 and 80.8% in 2001 ($P < 0.0001$). The proportion of arginine-requiring isolates increased from

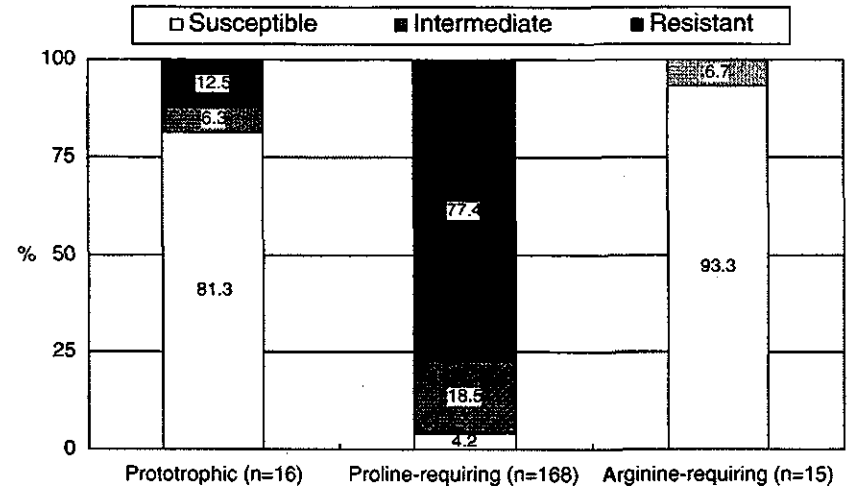
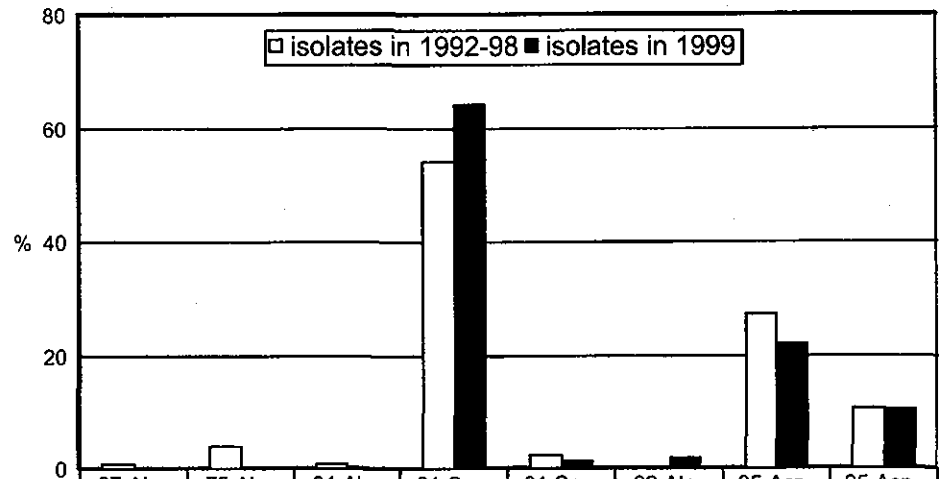


Fig. 4. Relationship between auxotype and ciprofloxacin susceptibility in *N. gonorrhoeae* isolates.

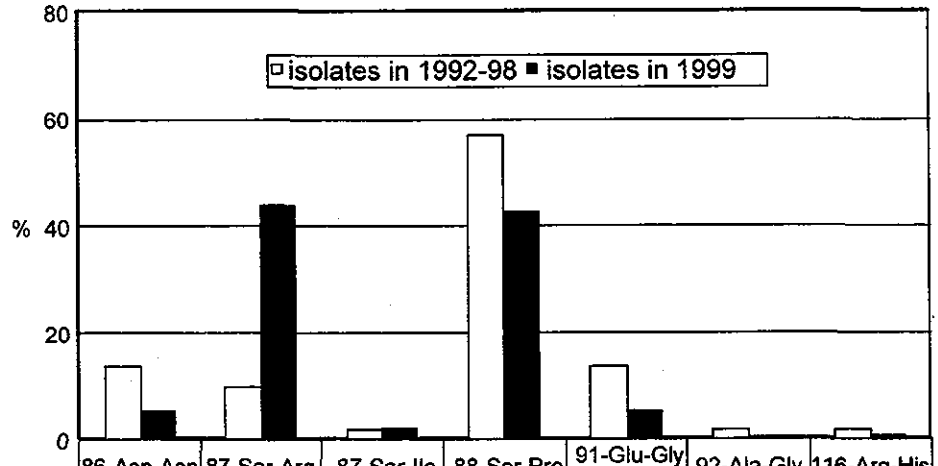


	67-Ala-Ser	75-Ala-Ser	84-Ala-Pro	91-Ser-Phe	91-Ser-Cys, Tyr	92-Ala-Pro	95-Asp-Asn	95-Asp-Gly
□ isolates in 1992-98	0.8	4	0.8	54	2.4	0	27.4	10.5
■ isolates in 1999	0	0	0	64	1.3	1.9	22	10.4

Fig. 5. Comparison of amino acid substitution within QRDR in the GyrA protein between gonococcal isolates in 1992–1998 and 1999.

3.3% in 1992–1993 to 25.9% in 1995–1996 and then decreased to 7.2% in 2002. These data suggest that an association exists between the increase in the proportion of fluoroquinolone-resistant gonococci and the increase in the proportion of proline-requring isolates.

We then examined the association between gonococcal auxotype and ciprofloxacin susceptibility in 208 gonococcal isolates in 2001. Significant differences were seen in the susceptibility to ciprofloxacin among the auxotypes. The results are shown in Fig. 4. Of 168 proline-requiring isolates, 130 (77.4%) were resistant to ciprofloxacin, 31 (18.5%) were intermediate and only the remaining 7 (4.2%) were susceptible. Of 15 arginine-requiring isolates, no isolate was resistant to ciprofloxacin, 1 (6.7%) was intermediate and the remaining 14 (93.3%) were susceptible. Of 16 prototrophic isolates, 2 (12.5%) were resistant to ciprofloxacin, 1 (6.3%) was intermediate and the remaining 13 (81.3%) were susceptible. These results indicated that the proline-requiring isolates were more resistant to fluoroquinolone than the prototrophic isolates or arginine-requiring isolates. Proline-requiring isolates are generally less susceptible to antibiotics such as penicillins or cephalosporins than prototrophic isolates. This



	86-Asp-Asn	87-Ser-Arg	87-Ser-Ile	88-Ser-Pro	91-Glu-Gly Lys, Gln	92-Ala-Gly	116-Arg-His
□ isolates in 1992-98	13.7	9.8	2	56.9	13.7	2	2
■ isolates in 1999	5.5	43.7	2.2	42.6	5.5	0	0.5

Fig. 6. Comparison of amino acid substitution within QRDR in the ParC proteins between gonococcal isolates in 1992–1998 and 1999.

has been reported previously for non-PPNG [13,14] and in PPNG [15]. Therefore, proline-requiring gonococcal isolates may acquire chromosomal-mediated resistance not only to β-lactams but also to structurally unrelated fluoroquinolones than do non-requiring isolates.

3.9. Substitution in GyrA and ParC

We also investigated the genetic alterations within QRDR in the *gyrA* and *parC* genes in the isolates susceptible or resistant to ciprofloxacin. The frequently identified substitutions were a serine to phenylalanine substitution at position 91 (Ser-91 in *N. gonorrhoeae* GyrA corresponds to Ser-83 in *Escherichia coli* [16]) and an aspartic acid to asparagine or glycine substitution at position 95 within GyrA in both isolates of 1992–1998 and1999 (Fig. 5). The frequently identified substitutions were a serine to proline substitution at position 88, an aspartic acid to asparagine substitution at position 86 and a glutamic acid to glycine, lysine or glutamine substitution at position 91 in isolates of 1992–1998. A serine to arginine substitution at position 87 and a serine to proline substitution at position 88 within ParC were identified in the isolates of 1999 (Fig. 6). Interestingly, of 87 isolates resistant to ciprofloxacin in 1999, 2 (2.3%) contained five amino acid substitutions, 30 (34.5%) contained four amino acid substitutions, 46 (52.9%) contained three amino acid substitutions, 7 (8.0%) contained two amino acid substitutions and 2 (2.3%) contained only one amino acid substitution within the GyrA and ParC proteins. High-level fluoroquinolone-resistant isolates are accumulating amino acid substitutions within the GyrA and ParC proteins.

4. Conclusion

In Japan, where high prevalence of fluoroquinolone-resistant isolates and increasing prevalence of multidrug-resistant isolates have been shown, ceftriaxone, cefixime or spectinomycin are recommended as the first-line treatment regimen for gonococcal infections. Fluoroquinolones should be avoided and the surveillance of antimicrobial resistance of *N. gonorrhoeae*, the evolution of high-level fluoroquinolone resistance and mutidrug resistance, should be continued in Japan.

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九州地域における薬剤耐性淋菌の分離状況に関する研究

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九州地域における薬剤耐性淋菌の分離状況に関する研究

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小松　潔⑧　山口　秋人⑧　占部　治邦⑧
天本　太平⑧　湯下　芳明⑧　徳永　毅⑧
南　祐三⑧　鈴　博司⑧　計屋　紘信⑧
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はじめに

淋菌感染症に対する抗菌化学療法においては，近年次々に開発されたいわゆるフルオロキノロン系薬（キノロン系薬）が，従来臨床の現場で問題になっていたペニシリン耐性株やテトラサイクリン耐性株を含めた *Neisseria gonorrhoeae*（淋菌）に強い抗菌力を示すことより，第一選択薬として汎用され，優れた臨床効果を示していた。しかしながら最近，淋菌のキノロン系薬に対する耐性化が大きな問題となっている[1]。さらに現在では，キノロン耐性淋菌に抗菌力を示していた経口セフェム系薬に対する淋菌の耐性化も進行している[2]。これまで我が国における薬剤耐性淋菌の分離状況に関する研究は，限られた一部の地域で分離された少数株を対象にしたものが多く，広い地域における多数株を対象にしたものは少ない。そこで，今回我々は九州地域の5県から分離した718株の淋菌を対象に，各種薬剤に対する感受性を測定し，耐性淋菌の分離状況を検討した。

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Ⅰ．対象と方法

1．淋菌株

対象淋菌株は，2000年10月から2002年4月までに九州地域の5県において男性尿道炎患者から分離同定された718株であった。地域別の分離株数は福岡県303株，長崎県119株，熊本県67株，鹿児島県181株，および沖縄県48株であった。淋菌株の収集は，尿道分泌物を綿棒で採取して Thayer-Martin 選択培地（日本 Becton Dickinson）に接種した後，淋菌輸送用の CO_2 培養パック（Bio-Bag Environmental Chamber Type C，日本 Becton Dickinson）に入れ，三菱化学ビーシーエル化学

療法研究室に郵送することにより行った。淋菌の同定はグラム染色性を確認後，ゴノチェック-Ⅱキット（コスモ・バイオ）で行った。収集同定された淋菌株は，薬剤感受性測定まで－80℃で凍結保存した。

2. 薬剤感受性測定

各種薬剤の最小発育阻止濃度（minimal inhibitory concentration：MIC）値の測定は，米国National Committee for Clinical Laboratory Standards (NCCLS) 法に準じて寒天平板希釈法で行った[3]。接種菌量は10^6 cfu/mLとし，β-lactamase産生能の測定，すなわちpenicillinase-producing *N. gonorrhoeae*（PPNG）の検出は，β-チェック（日本生物材料センター）で行った。使用薬剤は，キノロン系のシプロフロキサシン，レボフロキサシン，シタフロキサシン，ペニシリン系のペニシリンG，アモキシシリン・クブラン酸，テトラサイクリン系のテトラサイクリン，セフェム系のセフィキシム，セフポドキシム，フトリアキソン，セフォジジム，およびアミノグロコシド系のスペクチノマイシンの合計11薬剤であった。

3. 薬剤耐性淋菌の判定

各種薬剤耐性淋菌の判定は米国NCCLS法の基準に準じて行った[3]。すなわち，キノロン耐性はシプロフロキサシンのMIC値≧1 μg/mL，中等度耐性はMIC値が0.12～0.5 μg/mL，感受性はMIC値≦0.06 μg/mLであった。ペニシリン耐性はペニシリンGのMIC値≧2 μg/mL，中等度耐性はMIC値が0.12～1 μg/mL，感受性はMIC値≦0.06 μg/mLであった。テトラサイクリン耐性は同薬のMIC値≧2 μg/mL，中等度耐性はMIC値が0.5～1 μg/mL，感受性はMIC値≦0.25 μg/mLであった。セフェム系に関してはセフィキシムが標準薬の1つとされている[4]が，本薬剤における明確な耐性の基準がない。そこで今回の研究では，セフィキシム耐性は同薬のMIC値≧0.5 μg/mL，中等度耐性はMIC値が0.12～0.25 μg/mL，感受性はMIC値≦0.06 μg/mLとした。スペクチノマイシン耐性は同薬のMIC値≧128 μg/mL，中等度耐性はMIC値が64 μg/mL，感受性はMIC値≦32 μg/mLであった。

Ⅱ. 結　　果

1. 九州地域5県における各種耐性淋菌の分離状況と各種薬剤のMIC値

1）各種耐性淋菌の分離状況

九州地域5県より収集された718株の淋菌における各種薬剤に対する耐性株，中等度耐性株，感受性株の分離頻度を図1に示した。キノロン耐性株は56.5%，中等度耐性株は22.3%であった。ペニシリン耐性株は27.8%，中等度耐性株は61.6%であった。なお，PPNGはわずか5株のみが分離され，その分離頻度は0.7%であった。テトラサイクリン耐性株は23.1%，中等度耐性株は57.0%であった。セフィキシム耐性株は0.7%，中等度耐性株は45.3%であった。スペクチノマイシンに対しては全てが感受性株であった。このようにキノロン耐性株の分離頻度の高さが目立った。なお，セフィキシム耐性株の分離頻度は低かったものの，中等度耐性株の分離頻度は高かった。

2）各種薬剤のMIC値

表1に各種薬剤の淋菌に対するMIC値を示した。シタフロキサシンを除くキノロン系のMIC値は他系統に比べ高く，シプロフロキサシンのMIC50値，MIC90値はそれぞれ2 μg/mL，32 μg/mLであった。また，レボフロキサシンのMIC50値，MIC90値もそれぞれ2 μg/mL，8 μg/mLと

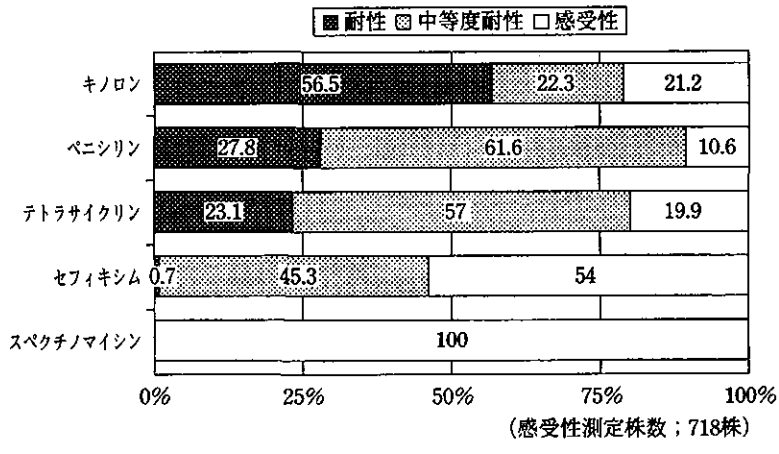


図　1　　九州地域における薬剤耐性淋菌の分離状況

表 1　九州地域で分離された淋菌に対する各種薬剤のMIC値

薬剤	MIC (μg/mL)		
	50%	90%	範囲
シプロフロキサシン	2	32	≤0.001～64
レボフロキサシン	2	8	0.002～16
シタフロキサシン	0.06	0.25	≤0.001～0.5
ペニシリンG	1	2	0.004～8
アモキシシリン・クラブラン酸	1	2	0.03～4
テトラサイクリン	1	2	0.015～4
セフィキシム	0.03	0.25	≤0.001～0.5
セフポドキシム	0.12	2	≤0.001～4
セフトリアキソン	0.03	0.06	≤0.001～0.25
セフォジジム	0.06	0.12	≤0.001～0.5
スペクチノマイシン	8	16	1～16

感受性測定株数；718株

高かった。ペニシリンGのMIC50値，MIC90値はそれぞれ1μg/mL，2μg/mLであった。アモキシシリン・クラブラン酸のMIC値はペニシリンGと同じ値であった。また，テトラサイクリンのMIC50値，MIC90値も同様にそれぞれ1μg/mL，2μg/mLであった。今回薬剤感受性測定を行った薬剤においては，セフェム系が優れた抗菌力を示した。なかでもセフトリアキソンの抗菌力が最も優れ，そのMIC50値，MIC90値はそれぞれ0.03μg/mL，0.06μg/mLであった。またセフォジジム，セフィキシムの抗菌力も他系統の薬剤に比べ優れていた。スペクチノマイシンのMIC50値，MIC90値はそれぞれ8μg/mL，16μg/mLであった。

2. 耐性淋菌の分離頻度とMIC値の地域別比較

1）キノロン系薬

キノロン耐性株の分離頻度は，熊本県68.7%，長崎県65.6%，沖縄県58.3%，福岡県57.8%，鹿児島県43.7%の順に高かった。このようにキノロン耐性株の分離頻度は熊本県が最も高く，鹿児島県が最も低かった（図2）。シプロフロキサシンのMIC値の比較では，鹿児島県分離株に対するMIC50値（0.25μg/mL）は，長崎県と熊本県分離株に対するMIC50値（4μg/mL）の1/16，また福岡県と沖縄県分離株に対するMIC50値（2μg/mL）の1/8の値であった。なお，MIC90値においてはほとんど地域差がなかった。レボフロキサシンとシタフロキサシンのMIC値の比較でも鹿児島県分離株に対するこれら薬剤のMIC50値は，他県の分離株に対する値に比べ低かった（表2-a）。

2）ペニシリン系薬

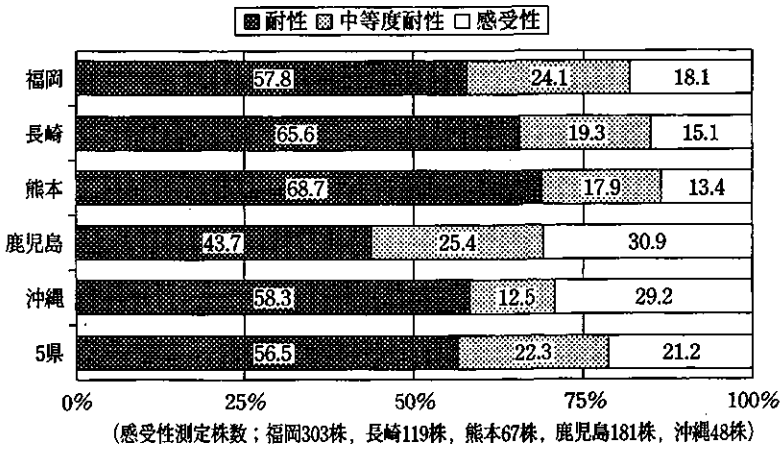
ペニシリン耐性株の分離頻度は，沖縄県35.4%，鹿児島県32.0%，長崎県27.7%，福岡県26.1%，熊本県19.4%の順に高かった。このようにペニシリン耐性株の分離頻度は沖縄県が最も高く，熊本県が最も低かった（図3）。PPNGが分離されたのは長崎県のみで，その分離頻度は4.2%と低い値であった。ペニシリンGのMIC50値とMIC90値に地域差はなかった（表2-a）。

3）テトラサイクリン系薬

テトラサイクリン耐性株の分離頻度は，鹿児島県29.3%，長崎県26.1%，福岡県20.1%，沖縄県18.7%，熊本県17.9%の順に高かった。このようにテトラサイクリン耐性株の分離頻度は鹿児島県が最も高く，熊本県が最も低かった（図4）。テトラサイクリンのMIC50値とMIC90値に地域差はほとんどなかった（表2-a）。

4）セフェム系薬

セフェム系においてはセフィキシム耐性株が福



（感受性測定株数；福岡303株，長崎119株，熊本67株，鹿児島181株，沖縄48株）

図 2　キノロン耐性淋菌の分離頻度の地域別比較